Neurophysiological signature of gamma-hydroxybutyrate augmented sleep in healthy male volunteers may reflect biomimetic sleep enhancement: A randomized controlled trial

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Abstract: Gamma-hydroxybutyrate (GHB) is an endogenous GHB/GABAB receptor agonist, which has demonstrated potency in consolidating sleep and reducing excessive daytime sleepiness in narcolepsy. Little is known whether GHB's efficacy reflects the promotion of physiological sleep mechanisms and no study has investigated its sleep consolidating effects under low sleep pressure. GHB (50 mg/kg p.o.) and placebo were administered in 20 young male volunteers at 2:30 a.m., the time when GHB is typically given in narcolepsy, in a randomized, double-blinded, crossover manner. Drug effects on sleep architecture and electroencephalographic (EEG) sleep spectra were analyzed. In addition, current source density (CSD) analysis was employed to identify the effects of GHB on the brain electrical sources of neuronal oscillations. Moreover, lagged-phase synchronization (LPS) analysis was applied to quantify the functional connectivity among sleep-relevant brain regions. GHB prolonged slow-wave sleep (stage N3) at the cost of rapid eye movement (REM) sleep. Furthermore, it enhanced delta-theta (0.5-8 Hz) activity in NREM and REM sleep, while reducing activity in the spindle frequency range (13-15 Hz) in sleep stage N2. The increase in delta power predominated in medial prefrontal cortex, parahippocampal and fusiform gyri, and posterior cingulate cortex. Theta power was particularly increased in the prefrontal cortex and both temporal poles. Moreover, the brain areas that showed increased theta power after GHB also exhibited increased lagged-phase synchronization among each other. Our study in healthy men revealed distinct similarities between GHB-augmented sleep and physiologically augmented sleep as seen in recovery sleep after prolonged wakefulness. The promotion of the sleep neurophysiological mechanisms by GHB may thus provide a rationale for GHB-induced sleep and waking quality in neuropsychiatric disorders beyond narcolepsy.

DOI: https://doi.org/10.1038/s41386-019-0382-z

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Original investigation to be submitted to

Neuropsychopharmacology

Version of 11 April, 2019

Number of words in the abstract: 240

Number of words in the article: 430

Number of references: 69

Number of figures: 5

Number of tables: 0

Supplemental Material: yes

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Abstract

Gamma-hydroxybutyrate (GHB) is an endogenous GHB/$GABA_B$ receptor agonist, which has demonstrated potency in consolidating sleep and reduce excessive daytime sleepiness in narcolepsy. Little is known whether GHB's efficacy reflects the promotion of physiological sleep mechanisms, and no study has investigated its sleep consolidating effects under low sleep pressure.

GHB (50 mg/kg p.o.) and placebo were administered in 20 young male volunteers at 2:30 am, the time when GHB is typically given in narcolepsy, in a randomized, double-blinded, cross-over manner. Drug effects on sleep architecture and electroencephalographic (EEG) sleep spectra were analyzed. Additionally, current source density (CSD) analysis was employed to identify the effects of GHB on neuronal oscillations at the level of brain electrical sources. Moreover, lagged-phase synchronization (LPS) analysis was applied to quantify the functional connectivity between sleep relevant brain regions.

GHB prolonged slow wave sleep (stage N3) at the cost of rapid eye movement (REM) sleep. Furthermore, it enhanced delta-theta (0.5-8 Hz) activity in NREM and REM sleep, while reducing activity in the spindle frequency range (13-15 Hz) in sleep stage N2. The increase in delta power predominated in medial prefrontal cortex, parahippocampal and fusiform gyri, and posterior cingulate cortex. Theta power was particularly increased in the prefrontal cortex and both temporal poles. Moreover, in the theta range, the brain areas affected by GHB exhibited increased LPS among each other.

Our study revealed distinct similarities between GHB-augmented sleep and physiologically augmented sleep as seen in recovery sleep after prolonged wakefulness in healthy men. The promotion of the sleep neurophysiological mechanisms by GHB may, thus, provide a rationale for GHB-induced sleep and waking quality in neuropsychiatric disorders beyond narcolepsy.

Introduction

Sleep-wake disturbances are highly prevalent in society, especially among patients with neurological and psychiatric disorders (Fernandez-Mendoza and Vgontzas, 2013; Ohayon et al., 1998; Weissman et al., 1997). The restoration and promotion of physiological sleep represents a core aim in psychopharmacology. Benzodiazepines and z-substances are positive allosteric $GABA_A$ receptor modulators, which are frequently used to manage sleep disturbances (Wafford and Ebert, 2008; Winsky-Sommerer, 2009). Yet, their clinical efficacy is insufficient, most likely because the neural oscillations produced by those drugs distinctly differ from physiological sleep oscillations (Akeju and Brown, 2017). More precisely, benzodiazepines induce frontal alpha and beta oscillations, burst suppression and isoelectricity, indicating a disruption of sensory, cognitive and memory encoding processing circuits rather than a restoration of physiological sleep, which is why these drugs often cause neurocognitive deficits (Feinberg et al., 2000; Fritz et al., 2016; Patat et al., 1994; Soehle et al., 2015; Van Lier et al., 2004; Wafford and Ebert, 2008). Thus, therapeutic alternatives are urgently needed.

Gamma-hydroxybutyrate (GHB or sodium oxybate), an endogenous GHB/$GABA_B$ receptor agonist, has recently gained interest as a potential pharmacological agent to promote physiological sleep (for review, e.g. Mamelak, 2009). When administered at bedtime, GHB enhances sleep efficiency and electroencephalographic (EEG) slow waves in non rapid eye movement (NREM) sleep, and reduces pathological sleepiness in patients suffering from narcolepsy, Parkinson's disease and fibromyalgia (Huang and Guilleminault, 2009; Ondo *et al*, 2008; Swick, 2011; Van Cauter *et al*, 1997; Vienne *et al*, 2012; Büchele et al., 2018). Given the hypothesized importance of EEG slow waves in NREM sleep for cognition and emotions (Tononi and Cirelli, 2014; Walker, 2009), GHB's beneficial effects have primarily been assigned to its ability to promote slow wave sleep and to produce EEG slow oscillations during sleep (Godbout and Montplaisir, 2002; Morawska et al., 2016). Nevertheless, GHB also reduces cataplexies and hypnagogic hallucinations in narcolepsy, which may indicate that it normalizes rapid-eye-movement (REM) sleep (Godbout and Montplaisir, 2002), for example by reducing the number of awakenings during REM sleep episodes (Lapierre et al., 1990).

Despite the clinical observations summarized above, it is currently unclear whether GHB promotes physiological sleep mechanisms and enhances homeostatically regulated sleep intensity. Sleep homeostasis refers to the general biological principle that sleep is more intense than baseline sleep when recovering from prolonged waking or sleep restriction (Borbely, 1982). Sleep intensity can be reliably quantified in the EEG power spectrum during NREM sleep (Achermann and Borbély, 2017). More specifically, it is well established that EEG delta (~ 0.5-4.5 Hz) and theta (~ 6-9 Hz) activity in

NREM and REM sleep are enhanced over frontal brain sites after extended wakefulness, whereas activity in the frequency range of sleep spindles (~ 12-15 Hz) in NREM sleep is reduced (Bersagliere et al., 2017; Finelli et al., 2001). The reverse pattern is observed in the second half of a sleep episode, reflecting the declining trend of sleep propensity and sleep intensity throughout the night Moreover, the duration of stage N2 and REM sleep are reduced at the cost of stage N3 sleep. (Bachmann et al., 2012; Holst et al., 2014).

Because of its short half-life time, GHB in clinical practice is typically administered in two doses, one dose at bedtime and one dose in the middle of the sleep episode. In most previous studies in healthy volunteers, GHB was administered at the beginning of the night when sleep intensity is high (Van Cauter et al., 1997; Vienne et al., 2012). Here, we administered a therapeutic dose (50 mg/kg) of GHB to 20 healthy young men only once in the middle of the night and employed complementary neurophysiological imaging methods to test the hypothesis that the drug-induced effects on sleep and the sleep EEG mimic physiologically enhanced sleep intensity. Thus, we elucidated in detail the neurophysiological signature of GHB-augmented sleep, by analyzing the drug effects on sleep architecture, sleep EEG spectra, brain electrical sources, and functional connectivity among sleep relevant brain regions. To evaluate the similarities and differences of GHB-augmented sleep and naturally enhanced sleep, we compared our results with the neurophysiological changes that have been observed during recovery sleep after acute sleep deprivation in previous studies.

Methods

Permission. The study was approved by SwissMedic and Ethics Committee of the Canton of Zurich and registered at ClinicalTrials.gov (NCT02342366). All participants provided written informed consent according to the declaration of Helsinki.

Study design. The study followed a randomized, placebo-controlled, balanced, double-blind, cross-over design. All study participants completed a screening night in the sleep laboratory, to exclude sleep-related disorders such as sleep apnea (), restless legs syndrome (> 5 periodic limb movements per hour), sleep onset REM sleep () and insufficient sleep efficiency (< 80%) before definite enrollment into the study. The study protocol consisted of two randomized, experimental nights (GHB and placebo) separated by a washout phase of seven days. Each experimental night was preceded by an adaptation night for habituation to the laboratory environment.

Participants. Twenty healthy, male volunteers (mean age: 25.8 ± 5.1 years) completed the study. Following criteria were required for inclusion: (i) male sex (to avoid unknown pregnancy and a potential impact of menstrual cycle on primary outcome variables); (ii) age within the range of 18 to

40 years; (iii) absence of somatic or psychiatric disorders; (iv) no first-degree relatives with a history of heritable psychiatric disorders such as schizophrenia, bipolar disorder, autism, and ADHD; (v) non-smoker; (vi.) no history of regular drug use (lifetime use <5 occasions of each drug, except occasional cannabis use). No participant reported previous experiences with GHB in their life. Participants had to refrain from illegal drugs for two weeks and from caffeine for one week prior to the first experimental night and throughout the study. No alcohol was allowed 24 h before each study night. Participants were instructed to keep a regular sleep-wake rhythm with eight hours in bed from 23:00 to 07:00 during one week prior to the first experimental night and in the week between the two experimental nights. To ensure compliance with this requirement, participants wore an actimeter on the non-dominant arm and kept a sleep-wake diary. Both, actigraphical recordings and sleep wake diaries were qualitatively inspected by a skilled person prior the experimental nights to ensure adherence of the subject to the requested sleep-wake cycle. No subject had to be excluded due to violation of the instructed sleep schedule. Moreover, sleep efficiency during both adaptation nights, which preceded each experimental night, was found to be > 80%. All participants received a monetary compensation for study participation.

***Urine immunoassay*.** On each test night, urine samples were taken upon arrival in the laboratory, to ensure that all participants abstained from illegal drug use (Drug-Screen Multi 12-AE, Nal von Minden GmbH, Regensburg, DE).

Drug administration. Study volunteers sleeping in the sleep laboratory were awoken at 2:30 am to receive 50 mg/kg of GHB (Xyrem®; Cantonal pharmacy, Zurich, Switzerland) dissolved in 2 dl of orange juice or placebo, matched in appearance and taste. The administered dose represents the maximal therapeutic starting dose in narcolepsy. After GHB/placebo intake, volunteers where allowed to immediately return to sleep.

***EEG data acquisition*.** Sleep was quantified by all-night polysomnography with Rembrandt® Datalab (Version 8; Embla Systems, Planegg, Germany) from 23:00 (lights off) to 7:00 (lights on). The recording setup consisted of 19 EEG electrodes (Fp1, Fp2, F3, F4, F7, F8, Fz, T3, T4, T5, T6, C3, C4, Cz, P3, P4, Pz, O1, 02) according to the 10-20 system (Jasper, 1958), a bipolar electrooculogram (EOG), a submental electromyogram (EMG) and an electrocardiogram (ECG). All data were recorded with dedicated polygraphic amplifiers (Artisan®, Micromed, Mogliano Veneto, Italy). As in previous studies, the analog signals were conditioned by a high-pass filter (EEG: −3 dB at 0.15 Hz; EMG: 10 Hz; ECG: 1 Hz) and an antialiasing low-pass filter (−3 dB at 67.2 Hz), digitized and stored with a resolution of 256 Hz (sampling frequency of 256 Hz) (Rétey *et al*, 2006; Valomon *et al*, 2018).

Sleep stage scoring. For sleep scoring, the C3-A2 derivation was used. Sleep variables were visually scored based on 30-s epochs according to the criteria of the American Academy of Sleep Medicine (Iber C, Ancoli-Israel S, Chesson AL Jr. et al., 2007). Movement- and arousal-related artifacts were visually identified and excluded from analyses. The following sleep variables were computed: (i) duration of sleep stages (N1, N2, N3 and REM sleep, and wakefulness); (ii) duration of NREM sleep (time spent in stages N2 and N3); (iii) total sleep time (TST; time spent in N1, N2, N3 and REM sleep); (iv) time in bed (TIB; time between lights-off and lights-on); and (v) sleep efficiency index (SEI = [TST／TIB]*100). Sleep variables were computed for the 1st and 2nd halves of the sleep episodes, as well as for the entire night.

Spectral analysis. Power spectra were computed by a Fast-Fourier transform based on 4-s epochs (Hanning window, linear detrending, 50% overlap), resulting in a frequency resolution of 0.25 Hz. Spectra between 0.5-20 Hz were investigated. The average spectral power was computed across all epochs of a given sleep stage (N2, N3, NREM, REM), separately for the entire night, the 1st half and 2nd half of the night.

The power spectra were computed for the 1st and 2nd halves of the sleep episodes, as well as for the entire night, separately for NREM sleep, stages N2 and N3, and REM sleep.

Current Source Density (CSD) analysis. To investigate how GHB affected physiological EEG oscillations in NREM sleep, 10-min segments around the maximum of slow-wave activity values in the 1st NREM sleep episode following GHB/placebo administration were extracted for further analyses. All used segments were located in the peak phase of drug action (t_{45}-t_{75}) (Liechti et al., 2016). The procedure is depicted in Fig. 1, indicating the time point of GHB (red triangle) and placebo (black triangle) administration and segment extraction for CSD analysis (marked with a green arrow) in a representative individual. Extracted segments were pre-processed using Brain Vision Analyzer 2 software (Brain Products GmbH). First, EEG data were re-referenced to the average of all scalp electrodes. Second, a bandpass-filter from 0.5-40 Hz was applied to the EEG data, to attenuate channel drifts and satisfy the assumption of stationarity necessary for computing independent component analysis (ICA) (Onton and Makeig 2006). Third, movement-related artifacts were visually identified and excluded. Fourth, the preprocessed segment was segmented into 12-s segments and exported for further analysis. The minimal segment number used for CSD analyses was 45. Two subjects had to be excluded from CSD analysis because of insufficient data quality. The CSD was analyzed using exact low-resolution brain electromagnetic topography (eLORETA, http://www.uzh.ch/keyinst/loreta.htm), a widely used mathematical approach to estimate the sources of electrical currents measured with scalp EEG. eLORETA computes a three-dimensional EEG

CSD map by applying a three-spherical shell model restricting the solution space to grey matter and hippocampus, resulting in 6239 different voxels of 5 x 5 x 5 mm each. The anatomical brain model used for the computation of intracerebral CSD values was registered on a digitized average MRI brain of the Talairach and Tournoux atlas (Brain Imaging Centre, Montreal Neurological Institute). To compute spectral density ($\mu A/mm^2$), the signal was split into delta (0.5-4.5 Hz), theta (4.5-8 Hz), alpha (8-12 Hz), sigma (12-16 Hz) and beta (16-20 Hz) frequency bands.

Figure 1

Lagged Phase Synchronization (LPS) analysis. The LPS analysis implemented in the eLORETA software was used to characterize differences in functional connectivity among brain areas in GHB-augmented sleep when compared to placebo. Phase synchronization of neuronal oscillations has been repeatedly shown to be involved in coordinating the activity between distinct brain regions (Buzsáki and Draguhn 2004; Uhlhaas and Singer 2010). Thus, the quantification of LPS offers a valuable tool to investigate physiological processes underlying functional connectivity between distinct neuronal assemblies (Varela et al. 2001). This method has undergone cross-modal validation from both, diffusion tensor imaging (Babiloni et al., 2011) and fMRI (De Ridder et al., 2011). LPS allows calculating the functional connectivity among regions of interest (ROI) for all defined frequency bands. Six brain regions, which were significantly affected by GHB (as revealed by the CSD analysis) were chosen for ROI analyses: anterior cingulate cortex (ACC; X = 1, Y = 29, Z = 14), posterior cingulate cortex (PCC; X = 1, Y = -51, Z = 8), left dorsolateral prefrontal cortex (ldlPFC; X = -39, Y = 40, Z = 28), right dorsolateral prefrontal cortex (rdlPFC; X = 39, Y = 40, Z = 28), left parahippocampal gyrus (lPHG; X = -27, Y = -40, Z = -14), and right parahippocampal gyrus (rPHG; X = 27, Y = -40, Z = -14).

Statistical analyses*.* A linear mixed effects model, with *condition* (GHB vs. placebo) as within-subject factor and *subject* ID as random effect was employed on *R* (RStudio Version 1.0.136; RStudio, Inc.) for the analyses of the sleep variables (R-package 'lme4', Version 1.1-15). P-values of posthoc tests (R-package 'emmeans', Version 1.2.1) were corrected for multiple comparison using Benjamini-Hochberg correction of the false discovery rate (Hochberg and Benjamini, 1990). Spectral data were statistically analyzed with a linear mixed-effects model (R-package 'nlme'; Version 3.1), while controlling for inter-frequency bin correlations, by an autoregressive moving average model (ARMA)

and comparing bin-wise with general linear hypothesis tests. Statistical differences in CSD between the conditions were calculated using a nonparametric mapping approach as implemented in the eLORETA software (Pascual-Marqui et al. 2011). A voxel-by-voxel-dependent t-test was used, whereby the contrast between the conditions for all frequency bands were calculated separately. Corrections for multiple comparisons were performed across voxels and frequency bands, applying a randomization strategy with 5000 permutations. Moreover, statistical regularization (variance smoothing parameter for t-statistic at 5%) was applied.

Statistical differences in LPS between the conditions were calculated using a non-parametric mapping approach (as implemented in the eLORETA software) (Pascual-Marqui *et al*, 2011). A paired t-test was used, calculating the contrasts between the conditions for all frequency bands. Corrections for multiple comparisons were performed across ROIs and frequency bands, applying a randomization strategy with 5000 permutations.

Results

Sleep variables. GHB robustly prolonged N3 sleep and NREM sleep and shortened REM sleep when compared to placebo (Fig. 2). While these effects were significant when the entire night was considered (all $p<0.05$), they were even more pronounced when the analysis was restricted to the 2nd half of the night (all $p<0.001$; supplementary Table S1). No differences ($p>0.05$) between GHB and placebo were observed for sleep efficiency, the durations of total sleep time, duration of N1 and N2 sleep and wakefulness.

Figure 2

EEG power spectra. Sleep architecture and the evolution of EEG power between 0.5-30 Hz as well as EEG slow-wave activity (SWA; power in the 0.75-4.5 Hz range) in a representative individual in the placebo and GHB conditions are plotted in Fig. 1. Reflecting the declining trend in sleep intensity across the sleep episode, SWA in the second half of the night is drastically reduced compared to the first half of the night in the placebo condition. While the volunteer returned to sleep almost immediately following both GHB and placebo administration, a prominent GHB-induced increase in SWA in NREM sleep episodes 3 (at around 03:00) and 4 (at around 05:00) is clearly apparent. On the group level, the compound increased EEG activity in virtually all bins of delta/theta (0.5-8 Hz) frequencies in stages N2 and N3, as well as when these stages were analyzed together (Fig. 3). In N2

sleep and combined stages N2 & N3 (NREM sleep), GHB also reduced power in the frequency range of sleep spindles (13-15 Hz). In REM sleep, the differences between GHB and placebo were restricted to the < 1-Hz and 6-8 Hz bands. The GHB-induced changes in sleep EEG power spectra reflect more intense sleep when compared to placebo.

Figure 3

Current source density. CSD analysis in the 1st NREM sleep episode following GHB and placebo intake was performed for the EEG delta, theta, alpha, sigma and beta ranges (see Fig. 1 for an illustration of time point when the CSD analyses were conducted). These analyses revealed increased CSD in delta (0.5-4.5 Hz) and theta (4.5-8 Hz) ranges following the administration of GHB when compared to placebo. The increase was distributed approximately symmetrically in both hemispheres. In the delta range, the following brain areas were affected by the drug: parahippocampal gyrus, lingual gyrus, fusiform gyrus, PCC, ACC, cuneus, Medial Frontal gyrus, subcallosal gyrus, rectal gyrus, inferior frontal gyrus, orbital gyrus, superior frontal gyrus and middle frontal gyrus (Fig. 4, upper panel). In the theta range, the following brain areas were affected by the drug: medial frontal gyrus, ACC, superior frontal gyrus, orbital gyrus, rectal gyrus, middle frontal gyrus, inferior frontal gyrus, subcallosal gyrus, insula, superior temporal hyrus, middle temporal gyrus, uncus, parahippocampal gyrus, inferior temporal gyrus (Fig. 4, lower panel). Table S2 (supplementary material) summarizes the results of the statistical analyses, reporting significantly affected brain regions, the number of significant voxels within the brain regions, Talairach coordinates of the maximal t-value and the corresponding t- and p-values.

Figure 4

Lagged phase synchronization. As illustrated in Fig. 5, functional connectivity (or synchronization) in the theta range (4.5-8 Hz) was significantly increased after GHB when compared to placebo between ACC and PCC, ACC and lPHG, ACC and rdlPFC, ACC and ldlPFC, ldlPFC and PCC, and between rdlPFC and rPHG. Table S3 (supplementary material) summarizes the t- and p-values of the corresponding connections. No significant effects of GHB on functional connectivity were found in the delta, alpha, sigma or beta ranges (results not shown).

Figure 5

Discussion

Our study provides evidence that a therapeutic dose of GHB induces a biomimetic enhancement of sleep intensity when administered to healthy adults under low homeostatic sleep pressure. More specifically, 50 mg/kg GHB given at 2:30 am, at the time of typical dosing of GHB dose in narcolepsy treatment, increased the duration of deep NREM sleep (N3) at the cost of REM sleep. Furthermore, it enhanced EEG delta and theta activity in NREM and REM sleep, while reducing spindle frequency activity in more superficial N2 sleep. Source localization suggested that GHB augmented delta band activity in NREM sleep especially in the ACC, in the medio-prefrontal cortex, in both PHG, and in the PCC. In the theta band, the increase predominated in the prefrontal cortex, ACC and both temporal poles. The functional connectivity in this frequency range during GHB-augmented sleep was strengthened among bilateral dlPFC, ACC, PCC, and bilateral PHG. Collectively, the GHB-induced changes in sleep and the sleep EEG resemble the characteristics of recovery sleep after sleep deprivation.

Clinically, GHB is the first-line treatment of excessive daytime sleepiness and cataplexy in narcolepsy type-1 and has recently been shown to be an effective novel treatment option for sleep-wake disturbances in Parkinson's disease (Büchele et al., 2018; Mamelak, 2018). Although it has been suggested that GHB may augment the regenerative functions of sleep (Mamelak, 2007b, 2009) and activate similar physiological mechanisms as sleep deprivation (Morawska et al., 2016), the neurophysiological signature of GHB-augmented sleep has not yet been systematically investigated. In fact, the two available studies that examined the effects of GHB on the sleep EEG in healthy volunteers in conditions of either attenuated or enhanced physiological sleep came to different conclusions. While one noted that GHB-induced slow waves are functionally dissimilar to physiological slow waves (Vienne et al., 2012), the other indicated that enhancing slow wave sleep with GHB reduces the homeostatic response to sleep loss and, thus, promotes physiological sleep-wake mechanisms (Walsh et al., 2010). Although in our study, no experimental manipulation of homeostatic sleep propensity was performed, the here shown findings support the latter hypothesis.

The prevalence of slow wave sleep, as well as EEG delta/theta frequencies in NREM sleep decrease in the course of a sleep episode (see Fig. 1), are reduced after a daytime nap, and predictably enhanced after extended wakefulness (Ong et al., 2017). It is widely accepted that recovery sleep following prolonged wakefulness is physiologically intensified when compared to baseline sleep. Here, at a time of reduced sleep pressure in the second half of a sleep episode, we observed striking

similarities between the effects of GHB and those induced by sleep deprivation. Following GHB intake, the duration of deep NREM sleep (stage N3) was increased, whereas REM sleep duration was reduced. Moreover, GHB increased delta and theta activity and attenuated spindle frequency activity in NREM sleep, and also enhanced theta power in REM sleep. Very similar changes are well established after sleep deprivation (Bersagliere et al., 2017; Borbely, 1982; Finelli et al., 2001; Maric et al., 2017). The CSD analysis further corroborated the similarity with the physiological effect, and revealed striking topographical overlaps between GHB-modulated and recovery sleep, including increased delta and theta power over frontal recording sites in NREM sleep (Borbély *et al*, 1981; Finelli *et al*, 2001; Marzano *et al*, 2010).

On a molecular level, most of GHB's sleep electrophysiological effects can most likely be attributed to low-affinity agonistic binding to $GABA_B$ receptors (Kaupmann et al., 2003), and work in mice, rats and cats suggests that $GABA_B$ receptors contribute to endogenous sleep oscillations (Gauthier et al., 1997; Juhász et al., 1994; Vienne et al., 2010). Given the wide-spread expression of $GABA_B$ receptors throughout the brain (Bowery et al., 1987), the area-specific increase in delta/theta power may appear astonishing. Nevertheless, GHB may predominantly increase low-frequency power in NREM sleep in brain areas, which are also increased under conditions of elevated homeostatic sleep pressure (Finelli et al., 2001; Marzano et al., 2010). On the other hand, while the present study is consistent with experimental and theoretical evidence that agonism at $GABA_B$ receptors increases deep sleep and EEG delta power (Vienne et al., 2010; Walsh et al., 2010), it cannot be excluded that also other neurotransmitter systems contribute to the promotion of EEG slow waves by GHB. For example, both GHB and $GABA_B$ receptors affect the serotonergic system (Szabo *et al*, 2004; Olpe et al, 1988), which also contributes to changes in the EEG spectrum which are similar to those seen after sleep deprivation (Landolt et al., 1999).

Apart from frontal-central sites, GHB enhanced delta activity in the PHG, the fusiform gyrus and the PCC. The functional connectivity analysis further revealed strengthened LPS among these areas after drug intake. A similar effect was found in a previous study, when investigating the effects of GHB on waking resting state LPS in healthy subjects (Von Rotz *et al*, 2017). Nevertheless, in waking subjects GHB's effects on CSD distinctly differ from those found in this study, indicating that GHB's neurophysiological effects may depend on the subject's state of consciousness.

In vitro studies demonstrated that bath application of GHB to thalamo-cortical neurons hyperpolarized these cells into a voltage range, -65 to -75 mV, at which the rhythmic pacemaker oscillations of the cell membrane exhibits a frequency of 0.5 to 4.0 Hz (Crunelli and Leresche, 2002; Williams et al., 1995). A similar hyperpolarization was also observed in hippocampal neurons, which might give a mechanistic explanation for the increased delta power in the PHG (Xie and Smart,

1992).

Intriguingly, these structures not only share tight anatomical connections, but are also functionally associated in the process of learning, memory encoding and retrieval (Born and Wagner, 2009; Maddock et al., 2001). Consequently, damages within these structures – as found in Alzheimer's disease – severely affect learning and memory (Köhler et al., 1998; Leech and Sharp, 2014). Likewise prefrontal atrophy in elderly and Alzheimer's patients was associated with hippocampal-dependent memory impairment and disrupted NREMS (Mander et al., 2013). Thus, GHB's ability to induce slow oscillations and strengthen functional connectivity in those brain sites, might underline its clinical potential as regenerative sleep-aid in neurodegenerative disorders (for review, e.g. Mamelak, 2007b, 2018).

GHB not only affected NREM sleep, but also had a significant impact on REM sleep. In accordance with previous research (Walsh et al., 2010; Vienne et al., 2012), GHB acutely reduced REM sleep and increased EEG delta and theta activity also in this sleep state. Despite its nominal reduction, more than one hour of REM sleep persisted during the peak phase of pharmacological action, indicating that REM sleep can prevail under elevated $GABA_B$-ergic tone, which may favor EEG-defined deep NREM sleep. This finding may reflect the well-known sleep-dependent disinhibition of and high endogenous pressure for REM sleep in the early morning hours, at the time of GHB administration (Borbely, 1982; Czeisler et al., 1980; Dijk and Czeisler, 1995). Nevertheless, even in this condition of high endogenous REM sleep pressure, we found no support for the notion that the stimulation of $GABA_B$ receptors favors sleep onset REM sleep episodes that was suggested by previous work (Vienne et al., 2012). It is likely that in the present study, REM sleep was reduced as a result of increased N3 sleep duration, yet an *ad libitum* sleep opportunity would be required to study the acute and subacute effects of GHB on REM sleep expression.

Similar to the spectral changes in NREM sleep, the increased delta/theta power in REM sleep is reminiscent of the impact of prolonged waking on recovery sleep (Borbély et al., 1981; Brunner et al., 1990; Marzano et al., 2010). These changes may underlie GHB's ability to restore REM sleep functions in neuropsychiatric disorders, including narcolepsy and major depressive disorder (Mamelak, 2009). On the other hand, GHB was reported to reduce arousals during REM sleep episodes in healthy volunteers (Lapierre et al., 1990). Future studies should investigate the interaction between GHB and sleep deprivation, to more conclusively tackle the question whether the same mechanisms are activated during GHB-augmented sleep and recovery sleep after prolonged wakefulness. Although comparisons between pharmacologically and physiologically induced alterations of sleep mechanisms must be made with caution, several studies indicate that GHB-augmented sleep not only shows neurophysiological overlaps but also functional similarities

with physiological sleep. For example, acute GHB administration spared energy metabolism and, similar to well several wake-promoting neurotransmitter systems (Brancucci et al., 2004; Cruz et al., 2004; Hechler et al., 1991; Kuschinsky et al., 1985; Labouèbe et al., 2007; Szabo et al., 2004), induced growth hormone release in a similar fashion as natural slow-wave sleep (Van Cauter et al. 1997), and reduced the consequences of sleep loss on measures of alertness and attention (Walsh et al., 2010). Together with the present data, these convergent findings support the view, that GHB enhances restorative aspects of sleep rather than just inducing a sedative state. Interestingly, GHB seems to induce qualitatively uniform effects on several neurophysiological parameters across subjects. Anyhow, at the same time we observed relatively high inter-individual differences in the strength of the induced effects. Both pharmacokinetic and pharmacodynamics differences across subjects may account for this variance, including individual differences in drug metabolism, $GABA_B$ receptor density and distribution pattern of $GABA_B$ receptor isoforms (Cruz et al., 2004). Thus, further studies are needed to elucidate the mechanisms underlying these individual variations.

Limitations

When interpreting the current study, some limitations should be kept in mind. All study subjects where young healthy men, which limits the generalizability of the results to female, elderly or clinical populations. Moreover, the intervention comprised one single administration, whereas in a clinical setting, chronic administration is the rule. Thus, based on our findings, long-term GHB effects on sleep neurophysiology are difficult to predict so far. Finally, despite LORETA's accuracy in localizing cortical electrical sources, the algorithm is not able to detect subcortical sources. In other words, neural structures that are crucially involved in sleep-wake regulation, including thalamus, hypothalamus and basal ganglia, cannot be investigated with this method. Thus, it remains unclear whether and how these brain areas contribute to GHB's effects on sleep.

Conclusion

In the present study, a detailed sleep neurophysiological signature of GHB-augmented sleep under low-homeostatic sleep pressure was established. Intriguingly, the GHB-induced changes in sleep architecture, sleep EEG power spectra, current source density and functional connectivity mimicked many characteristics of physiologically intensified sleep, such as in recovery sleep following sleep deprivation. Thus, GHB's potential to promote physiological sleep mechanisms and its clinical application to consolidate sleep and improve waking quality in neurological and neuropsychiatric disorders should be further investigated.

Funding and disclosure:

All authors report no conflicts of interest, financial or otherwise. The study was supported by grants from the Swiss National Science Foundation (SNSF) to HPL (grant # 320030_163439) and the Clinical Research Priority Program *Sleep & Health* of the University of Zurich. Dario Dornbierer had full access to all data of the study and takes responsibility for their integrity and the accuracy of the data analyses.

Acknowledgements:

The authors thank Vinnie Kandra for her dedicated assistance in data collection and participant recruitment. Moreover, we thank Antonio Fernandez Guerrero for his valuable comments on the CSD and LPS analyses.

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